J. Soc. Cosmetic Chemists, **16**, 349–358 (1965)

Pyridoxine-3, 4-Diacylates and Their Use in Cosmetics

HARUYASU OHTA, M.Sc.*

Presented before the Third Congress of the I.F.S.C.C., June 21–26, 1964, New York City

Synopsis—Pyridoxine-3,4-diacylates can be prepared by a new process in good yield and high purity. Data are presented to demonstrate the oil solubility and the heat and light stability of these esters. Acute and chronic toxicities of these esters indicate that they are suitable for inclusion in cosmetics. It is further shown that the esters are hydrolyzed by tissue homogenates at rates which are influenced by the acid moiety used for esterification. Finally, clinical data are presented to demonstrate the utility of pyridoxine-3,4-dipalmitate in dermal therapy.

Introduction

Vitamin B_6 is one of the most important factors for nutrition and for health and beauty of the human skin. Hence, many attempts have been made to use vitamin B_6 as a cosmetic additive. Pyridoxine hydrochloride, which is a commercially available form of vitamin B_6, is not suitable for topically applied cosmetics because of its insolubility in oils and fats and its instability to heat and light. It is therefore desirable to prepare a heat- and light-stable, fat-soluble and percutaneously absorbable pyridoxine derivative for cosmetic use.

In 1956, Sakuragi and Kummerow (1–4) first synthesized long chain fatty acid triesters of pyridoxine and confirmed that they are sources of fat-soluble, heat- and light-stable and biologically active vitamin B_6 when applied to the rat.

In 1961, Rocheggiani (5) reported on the cutaneous actions of

* Nihon Surfactants Industries Co. Ltd., 29, 3-chome, Hasune-cho, Itabashi-ku, Tokyo, Japan.

TABLE I

Physical Properties of Pyridoxine-3,4-Diacylates

Pyridoxine Diacylate	M.P., °C	Formula	M.W.
Dibutyrate	57–58	$C_{16}H_{23}O_5N$	309.37
Dioctanoate	69–71	$C_{24}H_{39}O_5N$	421.58
Di-*iso*-octanoate	Liquid	$C_{24}H_{39}O_5N$	421.58
Dilaurate	79–80	$C_{32}H_{55}O_5N$	533.80
Dipalmitate	88–89	$C_{40}H_{71}O_5N$	646.01

TABLE II

Solubilities of Pyridoxine-3,4-dioctanoate

Solvent	g./100 g. at 25°C
Isopropyl myristate	2
Olive oil	1
Oleyl alcohol	3.5
Oleic acid	5
Liquid paraffin	0.07
70% ethanol	0.3 at $-5 \sim -10$°C
60% ethanol	0.1 at $-5 \sim -10$°C

TABLE III

Solubilities of Pyridoxine-3,4-dipalmitate

Solvent	g./100 g. at 25°C	g./100 g. at 75°C
Ethanol	0.5	
Isopropyl myristate	0.1	3
Olive oil	0.1	5
Oleyl alcohol	0.2	5.5
Oleic acid	0.1	5.5
Liquid paraffin	0.01	0.5

TABLE IV

Sunlight Stability of Pyridoxine Derivatives

Derivatives	Irradiation Time, hr.	Transmittance, %
Pyridoxine hydrochloride	0	99
	15	92
	30	87
Pyridoxine-3,4-dibutyrate	0	100
	15	99
	30	98
Pyridoxine-3,4-dioctanoate	0	100
	15	99.5
	30	98

pyridoxine tripalmitate. He concluded from a series of experiments that pyridoxine tripalmitate is very effective in keeping the human skin healthy and beautiful. The large-scale preparation of pyridoxine tripalmitate is not easy, nor is this compound satisfactory for cosmetic use because of its insufficient fat-solubility.

A new commercial method for the synthesis of heat- and light-stable and fat-soluble derivatives of pyridoxine, pyridoxine-3,4-diacylates, has been developed in this laboratory. Patents for this method of preparation are now pending in the United States of America, Great Britain, France, Switzerland, West Germany, and Japan.

The chemical and physical properties, biological activity, toxicity and effects on the skin of these diacylates have been examined. It appears that the higher fatty acid diesters are heat- and light-stable, fat-soluble and hydrolyzed into free pyridoxine *in vivo* and show the biological activity of vitamin B_6.

The present report deals mainly with the dibutyrate, dioctanoate, dilaurate, and dipalmitate of pyridoxine.

Experimental and Discussion

Materials

All the diesters used were white crystalline powders, except the di-*iso*-octanoate. Some of the properties of these diesters are tabulated in Tables I, II, and III.

Heat Stability

Two grams of pyridoxine-3,4-dipalmitate was dissolved in 10 g. of olive oil and heated for six hours at 150–160°C. At the end of this heating period, the oil was brown. The oil was then diluted with 100 ml. of petroleum ether and cooled in the refrigerator for two days. The precipitate was removed by filtration and washed with petroleum ether. The crystalline precipitate was pure white and weighed 1.6 g. The crystals showed no depression in melting point when mixed with authentic pyridoxine-3,4-dipalmitate. The dilaurate and dioctanoate were tested in a similar manner, and the recovered unchanged esters weighed 1.4 and 1.1 g. respectively. The dibutyrate, however, could not be recovered from the heated brown oil, presumably because most of the diester was destroyed by heat.

Light Stability

One gram of each of the pyridoxine derivatives was dissolved in 100 g. of 50% aqueous ethyl alcohol. Samples of these solutions were

TABLE V

LD_{50} of Pyridoxine-3,4-dipalmitate and Related Compounds (Mice)

Compound	Administration	LD_{50}, g./kg.	
		24 Hr.	7 Days
Pyridoxine-3,4-dipalmitate	Oral	7.1	5.1
	Subcutaneous	4.4	1.6
Pyridoxine hydrochloride	Oral	3.8	3.8
	Subcutaneous	1.7	1.4
Palmitic acid	Oral	5.0	4.2
	Subcutaneous	...	4.2

sealed into test tubes and placed into sunlight. The transmittance of the irradiated solutions was read at 435 mμ with a spectrophotometer. The data shown in Table IV indicate that the esters are not severely discolored during long exposure to sunlight and are more stable than pyridoxine hydrochloride.

*Toxicity**

Acute Toxicity

The acute toxicity of pyridoxine-3,4-dipalmitate was studied in mice and is recorded as LD_{50} in Table V. Pyridoxine hydrochloride and palmitic acid were also tested as control materials. The ester and palmitic acid were dissolved in ethanol and then mixed with propylene glycol. Pyridoxine hydrochloride was studied in an aqueous solution. The mixture and the solution of samples were administered orally and subcutaneously to mice.

From the results shown in Table V, it can be concluded that pyridoxine-3,4-dipalmitate is less toxic than pyridoxine hydrochloride.

Chronic Toxicity

Six groups each of five male and five female rats weighing from 85 to 135 g. were used in this test and fed for six months as follows:

Group A: Standard Diet
Group B: Standard Diet + 40 mg./kg./day of pyridoxine-HCl
Group C: Standard Diet + 50 mg./kg./day of palmitic acid

* This work was conducted at the Osaka City Institute of Hygiene (6).

Group D: Standard Diet + 7 mg./kg./day of pyridoxine-dipalmitate

Group E: Standard Diet + 70 mg./kg./day of pyridoxine-dipalmitate

Group F: Standard Diet + 700 mg./kg./day of pyridoxine-dipalmitate

Groups A, B, and C were control groups. The weights of the rats were recorded daily for six months, and the growth rate is shown in Fig. 1. When pyridoxine-3,4-dipalmitate was orally administered daily to rats (in an amount of $\frac{1}{1000}$ the oral LD_{50} for mice) for six

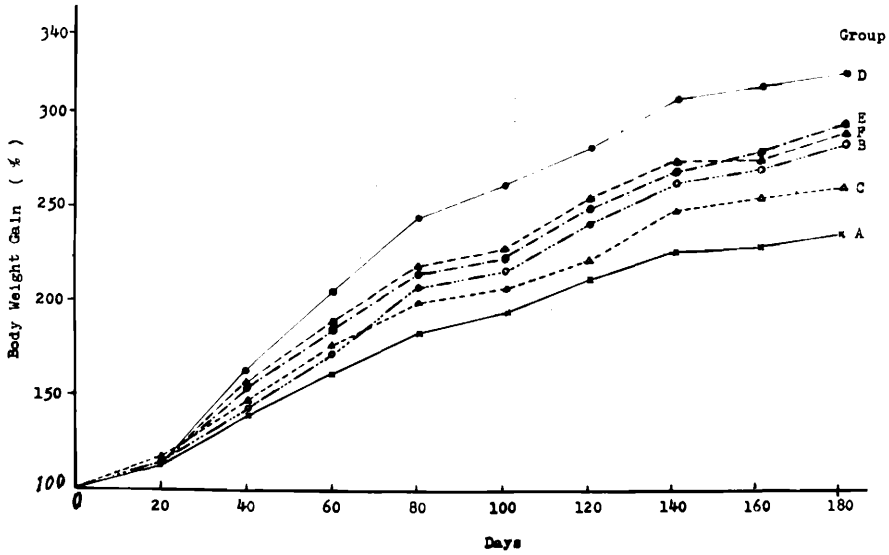

Figure 1.—Change in body weight of rats fed with various amount of pyridoxine-3,4-dipalmitate

months, the increase of the animal's weight was greater than when the equivalent amount of pyridoxine hydrochloride was administered. All rats used in this test survived during the test and were killed at the end of the test for observation of organs. No harmful effect of pyridoxine-3,4-dipalmitate was observed in organs at autopsy.

Percutaneous Absorption (*8*)

Rabbits weighing from 2 to 3 kg. were used for this test. An area of 5×15 cm.^{2} on the abdomens of the rabbits was clipped, and the hair was completely removed with a depilatory. After 24 hours, 6 g. of hydrophilic ointment containing one of the pyridoxine derivatives was

applied after ascertaining that there was no inflammation on the part to be tested. Blood samples were collected from the ear lobe by intravenous puncture and assayed for vitamin B_6 content.

The composition of the hydrophilic ointment is as follows:

Isopropyl myristate	1 g.
Cetanol	0.25 g.
Stearic acid	0.4 g.
Paraffin wax	1 g.
NIKKOL BL-9*	0.2 g.
NIKKOL BC-5†	0.15 g.
Mineral oil	1.35 g.
10% Triethanolamine solution	1ml.
Pyridoxine derivative	1%
Water to make	10 g.

In the preparation of the ointment containing pyridoxine hydrochloride, the triethanolamine solution was omitted from the above formulation.

The vitamin B_6 content in blood was determined as follows:

Standard curve: In the case of pyridoxine hydrochloride and of pyridoxine-3,4-dibutyrate, 1 ml. of an aqueous sample solution of known concentration (about 0.5 γ/ml.) was acidified with 6 ml. of 15% H_2SO_4, and the solution was heated at 100°C for one hour. After cooling, the pH of the solution was adjusted to 5.4, and the volume made up to 50 ml. with water. Samples of this solution (0.25, 0.5, 1 and 2 ml.) were pipetted into test tubes and diluted with water to 2.5 ml. After adding 2.5 ml. of culture medium and sterilizing at 100°C for 15 minutes, 1 drop of preincubated suspension of *Saccaromyces Carlsbergensis* culture was added, and the solution was incubated at 30°C for 20 hours. Absorbancy was then measured at 610 mμ, and a standard curve was constructed.

For the construction of the standard curve for pyridoxine-3,4-dioctanoate, 1 ml. samples of ethanolic solutions were hydrolyzed as mentioned above.

Pyridoxine assay in blood: A mixture of 1 g. of blood and 6 ml. of 15% H_2SO_4 was heated at 100°C for one hour. After cooling, the pH of the solution was adjusted to 5.4, and the liquid was centrifuged to remove blood pigments. The supernatant solution was separated, and its volume was adjusted to 50 ml. with water. The microbio-

* Polyoxyethylene lauryl alcohol ether. Nikko Chemicals Co., 1, 1 chome, Nihonbashi Bakurocho, Chiyoda-ku, Tokyo.

† Polyoxyethylene cetyl alcohol ether. Nikko Chemicals Co.

logical assay was carried out similarly as mentioned above, and the amount of pyridoxine was determined by reference to the standard curves.

The results are shown in Fig. 2. Pyridoxine and pyridoxine-hydrochloride were absorbed immediately, but their content in blood decreased rapidly. In contrast, the dibutyrate was also absorbed immediately, but the blood concentration remained at a constant level. The absorption of the dioctanoate and di-*iso*-octanoate was delayed, but the blood concentration increased gradually. The absorption of dilaurate and dipalmitate was not studied in this experiment. These

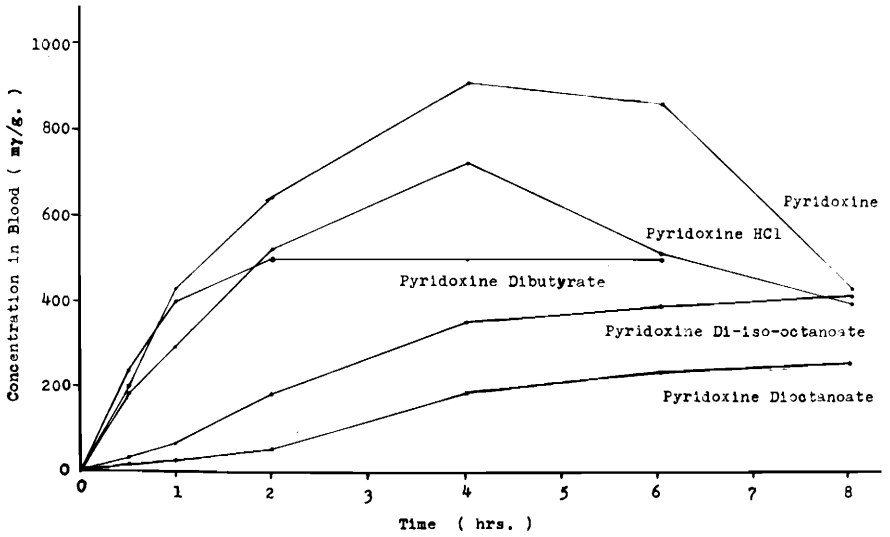

Figure 2.—Percutaneous absorption of pyridoxine and its derivative (1% in O/W cream base)

facts might suggest that cosmetics or ointments containing intermediate chain fatty acid diester of pyridoxine maintain higher levels of vitamin B_6 in the living body for a longer period of time than products containing pyridoxine.

Hydrolysis of Diacylates in Organs (9)

Liver, kidney, intestine, and blood of the mouse were used in this experiment. Liver, kidney, and intestine, from which as much blood as possible was removed, were washed with water. One gram of liver, 0.4 g. of kidney, 0.7 g. of intestine and 0.4 ml. of blood were homogenized with Tyrode's solution. These homogenates were poured into 50 ml. Erlenmeyer flasks, and the contents were adjusted to 7 g. with Tyrode's

TABLE VI

Hydrolysis of Pyridoxine-3,4-dibutyrate

Sample No.	Time for Hydrolysis	Amount Hydrolyzed, wt. %			
		Liver	Kidney	Intestine	Blood
1		105.8	98.9	98.9	80.1
2	2 hrs.	94.6	90.9	...	74.2
3		95.4	106.8	104.5	81.8
Average		98.6	98.8	101.7	78.7
1		106.6	108.0	92.8	70.4
2	1 hr.	...	97.5	107.6	69.6
3		98.4	98.9	104.4	66.2
Average		102.5	101.4	101.6	68.7
1		105.6	106.2	99.2	64.6
2	0.5 hr.	89.2	104.8	106.8	71.1
3		107.9	108.0	88.4	56.7
Average		100.9	106.3	98.1	64.1

TABLE VII

Hydrolysis of Pyridoxine-3,4-dioctanoate

Sample No.	Time for Hydrolysis	Amount Hydrolyzed, wt. %			
		Liver	Kidney	Intestine	Blood
1		91.5	82.3	82.2	0
2	2 hr.	87.6	69.2	82.3	0
3		101.7	79.0	87.2	0
Average		93.6	76.8	83.6	0
1		74.5	66.8	84.2	0
2	1 hr.	85.2	85.8	84.2	0
3		84.7	75.6	82.7	0
Average		83.9	76.1	80.2	0
1		63.1	72.4	64.1	0
2	0.5 hr.	62.9	61.6	59.8	0
3		84.0	76.4	79.9	0
Average		70.0	70.1	67.9	0

TABLE VIII

Hydrolysis of Pyridoxine-3,4-dilaurate

Sample No.	Time for Hydrolysis	Amount Hydrolyzed, wt. %		
		Liver	Kidney	Intestine
1		11.1	0	0.9
2	2 hr.	7.6	0	1.0
3		10.1	0	0.8
Average		9.6	0	0.9

solution. One ml. of one of the pyridoxine-3,4-diacylates solutions was added, and the flasks were shaken for 0.5, 1 or 2 hours at 37°C to hydrolyze the ester. After shaking, 3 ml. of 10% aqueous trichloroacetic acid solution was added, and the mixture was centrifuged. Five ml. of the supernatant was neutralized with 1.5% NaOH solution. For 1 ml. of the solution thus obtained, concentration of pyridoxine was determined by using 2,6-dibromoquinone chloroimide at 650 mμ.

The results are shown in Tables VI, VII, VIII, and IX. It can be seen that the dibutyrate was rapidly hydrolyzed in the blood and

TABLE IX

Hydrolysis of Pyridoxine-3,4-dipalmitate

Sample No.	Time for Hydrolysis	Amount Hydrolyzed, wt. %		
		Liver	Kidney	Intestine
1		0	0	0
2		2.3	0	0
3	2 hr.	1.3	0	0
4		0.4	0	0
Average		1.0	0	0

organ homogenates. The dioctanoate was not hydrolyzed in the blood but slightly in the organ homogenates. On the other hand, dilaurate and dipalmitate were not hydrolyzed even in the liver homogenate.

Dermal Therapy

During the clinical test, which was designed to demonstrate efficacy against several kinds of dermatitis, such as eczema and seborrhoea, hydrophilic ointments and lotions containing 0.2% and 0.02% pyridoxine-3,4-dipalmitate respectively were applied topically. The results showed that pyridoxine-3,4-dipalmitate was an effective treatment for skin diseases without any harmful side effects.

It is also noted that this substance showed marked antidandruff and anti-itching properties, as judged from the following studies. Fourteen dermal outpatients (7) suffering from eczemas and seborrhoea were treated with a hydrophilic ointment containing 0.2% pyridoxine-3,4-dipalmitate for seven to twenty days. This treatment was very effective in six cases, satisfactory in six cases and ineffective in two cases.

In three cases of seborrhoea and especially dermatitis of the face, patients felt a cooling sensation immediately after application; sebum vanished day by day. In one case of rosacea (of the first degree), reddening was reduced, oily luster vanished after ten days, and the disease was cured completely on the fourteenth day. In two cases of seborrhoea

capitis neonatorum, the sebum leaking scab was no longer observed after four days, and it was nearly cured in eight to ten days.

In two cases of facial pimples, no new ones developed after ointment application had begun, and the number of existing pimples decreased until none were observed at all. In two cases of desquamating eczema, scale decreased remarkably after four days, and the scale on the head and face almost vanished. Further, in two cases of eczema erythematosum and contact dermatitis, reddening decreased after three days.

In the two ineffective cases, the symptoms were those of serious cases of dermatitis; even oral or topical steroid hormone therapy (even in massive doses) was not fully effective, especially if the side effect induced by this hormone is considered.

Further, 162 cases of pityriasis simplex capitis and seborrhoea capitis were treated with a lotion containing ethyl alcohol, Tween 80* and 0.02% pyridoxine-3,4-dipalmitate. This lotion was effective in 75% of cases of scaling, in 77% of cases of itching and in 85% of cases of dry dandruff (10).

Summary

Pyridoxine-3,4-diacylates have been shown to be useful cosmetic ingredients.

The diesters of pyridoxine are considered to be more effective than the corresponding triesters since the former's content of the pyridoxine moiety is considerably larger.

When heat- and light-stability, fat-solubility, percutaneous absorbability and the effective content of pyridoxine are taken into account, pyridoxine-3,4-dioctanoate emerges as the most suitable derivative for cosmetic purpose.

(Received September 14, 1964)

References

(1) Sakuragi, T., and Kummerow, F. A., *J. Am. Chem. Soc.*, **78,** 839 (1956).
(2) Sakuragi, T., and Kummerow, F. A., *J. Am. Oil Chemists' Soc.*, **33,** 116 (1956).
(3) Sakuragi, T., and Kummerow, F. A., *J. Nutrition*, **58,** 557 (1956).
(4) Sakuragi, T., and Kummerow, F. A., *Arch. Biochem. Biophys.*, **63,** 32 (1956).
(5) Rocheggiani, G., *Soap, Perfumery Cosmetics*, **34,** 547 (1961).
(6) Osaka City Institute of Hygiene, reports in files of Nihon Surfactants Industries Co. Ltd.
(7) Dept. Dermatology, Osaka Medical College, report in file of Nihon Surfactants Industries Co. Ltd.
(8) Kamada, A., Personal communication.
(9) Kamada, A., Personal communication.
(10) Yasuda, T., *Japan. J. Dermatol.*, **73,** 487 (1963).

* Atlas Chemical Industries, Wilmington, Del.